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2003 MAY 5 PM 3:24

Additional Discussion of Reproductive / Developmental Toxicity for Silane, dichlorodimethyl-, reaction products with Silica, CAS RN 68611-44-9

In response to the EPA's recommendation that a combined reproductive / developmental toxicity study, such as OECD Test Guideline 421 be conducted, the consortium offers the following additional information in support of their position that no additional testing is needed nor required to understand the reproductive / developmental toxicity of the HPV Chemical Substance.

"Amorphous silica may be produced by a vapor-phase process yielding pyrogenic silica, or by a wet process, yielding either precipitated silica or silica gel. Pyrogenic amorphous silica is produced by the hydrolysis of silicon tetrachloride in an oxygen / hydrogen flame at temperatures of approximately 1000°C. The relatively high temperature yields a product that has a low water content and is noncrystalline in structure, i.e., amorphous. Precipitated silica and silica gel are manufactured by the precipitation of silicon dioxide from a solution of sodium silicate by the carefully controlled addition of an acid. Precipitated silica and silica gel contain a larger amount of bound water and tend to agglomerate, causing them to have a larger particle size. They also have an amorphous, non-crystalline structure.

All three types of silica may be surface-modified to produce silica that is hydrophobic, or water repelling, and all, when treated with dimethyldichlorosilane are registered under the Chemical Abstracts Service (CAS) name "Silane, dichlorodimethyl-, reaction products with silica, i.e., the HPV Chemical Substance.

A significant human exposure route to silica is through the diet, as silicas, in general, are widely used in cosmetics, foodstuffs, pharmaceuticals, and a wide variety of medical and dental applications. Various forms of silica are used as direct and indirect food ingredients. Both silicon dioxide and silica gel have been cleared by the United States Food and Drug Administration (FDA) for many food applications, as both a direct food additive at levels up to 2 percent by weight, and as a substance allowed in the manufacture of materials that come in direct contact with food in various producing, manufacturing, packing, preparing, transporting and holding operations. Pertinent sections of the regulations can be found in Section 21 of the Code of Federal Regulations, Part 172 Food Additives Permitted for Direct Addition to Food for Human Consumption. In addition, the FDA has affirmed in a letter to the Silica Trade Association that the use of silica gel in dietary supplements is generally recognized as safe (GRAS) (1993 Food Chemical News Guide, page 407).

When produced and handled in accordance with current good manufacturing practices pyrogenic and precipitated silicas, and silica gel meet the requirements for purity and quality for silicon dioxide described in the Food Chemical Codex, 4th Edition Effective July 1, 1996. In addition they also exceed the requirements of the United States National Formulary Official Monograph (NF18), the European Pharmacopoeia and other national pharmacopoeias including the Japanese Pharmacopoeia (Official Monograph for Part II / JP XIV) and the Deutsche Arzneibuch.

The EPA has also evaluated silicon dioxide and silica gel and found them to be of moderate to low toxicity. Consequently, they have been exempted from the requirement of a tolerance limit when applied to growing crops or agricultural commodities [40 CFR, § 180.1001(c)] or to animals [40 CFR, § 180.1001(e)]. Likewise the HPV chemical substance has also been exempted from the requirement of tolerance limit when applied to growing crops or agricultural commodities or to animals [40 CFR, § 180.1001(c) and (e)]. These clearances, all of which lead to human exposure to silica through the diet, support the view that the several uses of silicas as direct and indirect food ingredients are GRAS." (3)

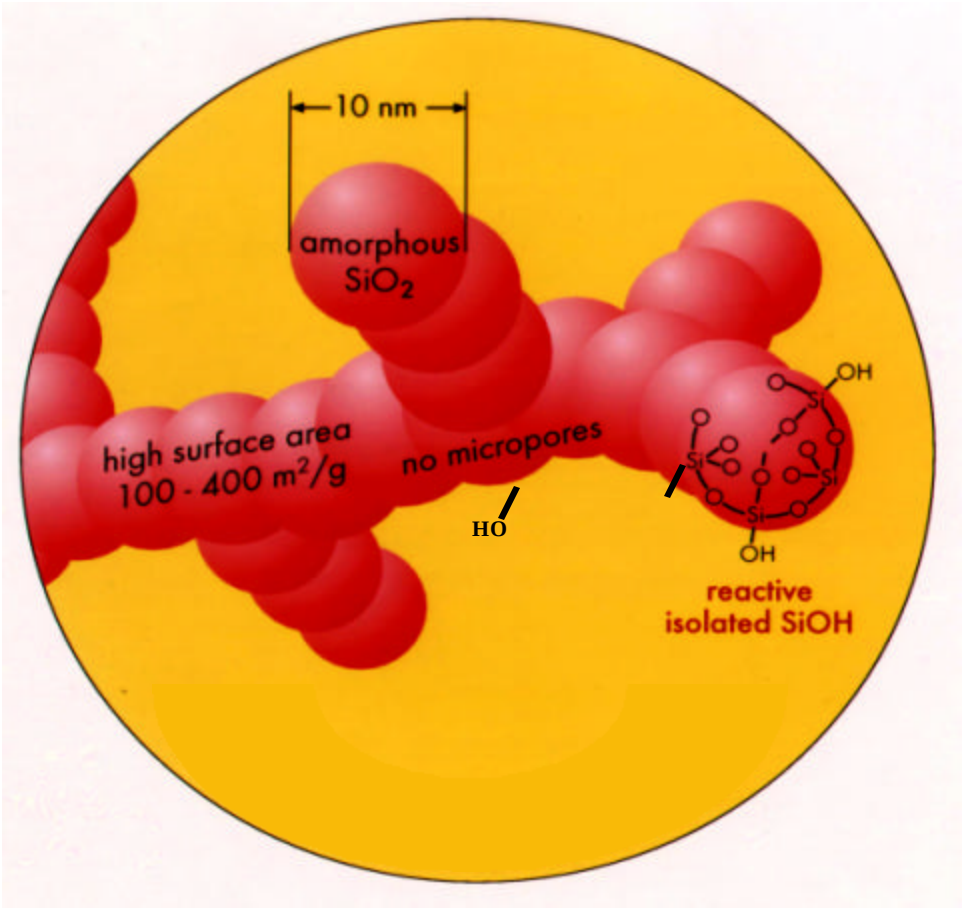
All of the three types of synthetic amorphous silica, that may be either hydrated or nonhydrated, contain silicon and oxygen connected in a three-dimensional macromolecule network. This

arrangement of atoms results in a large effective molecular weight and, except for the surface silanol groups imparts a general chemical inertness to all forms of silica.

There is significant physicochemical similarity between non-surface treated (hydrophilic) synthetic amorphous silica (Figure 1) and the HPV Chemical Substance, Silane, dichlorodimethyl-, reaction products with silica, (Figure 2). The hydrophilic nature of untreated synthetic amorphous silica is due to the presence of silanols (≡Si – OH) on the surface of the substance. When untreated synthetic amorphous silica is reacted with the treating agent dichlorodimethylsilane, the dichlorodimethylsilane first undergoes hydrolysis. Via condensation reactions short polydimethylsiloxane units are formed. The reactions are completed by the "backboning" of the OH terminated polydimethylsiloxane units with the surface silanols of the hydrophilic silica, again via condensation reactions. On the HPV Chemical Substance the original treating agent, dichlorodimethylsilane, is no longer detectable. The HPV Chemical Substance bears at its surface both the hydrophobic entities, (polydimethlysiloxanes), which project off the surface of the silica and somewhat shield the remaining hydrophilic entities, (the surface silanols), which had rendered the chemical substance initially hydrophobic.

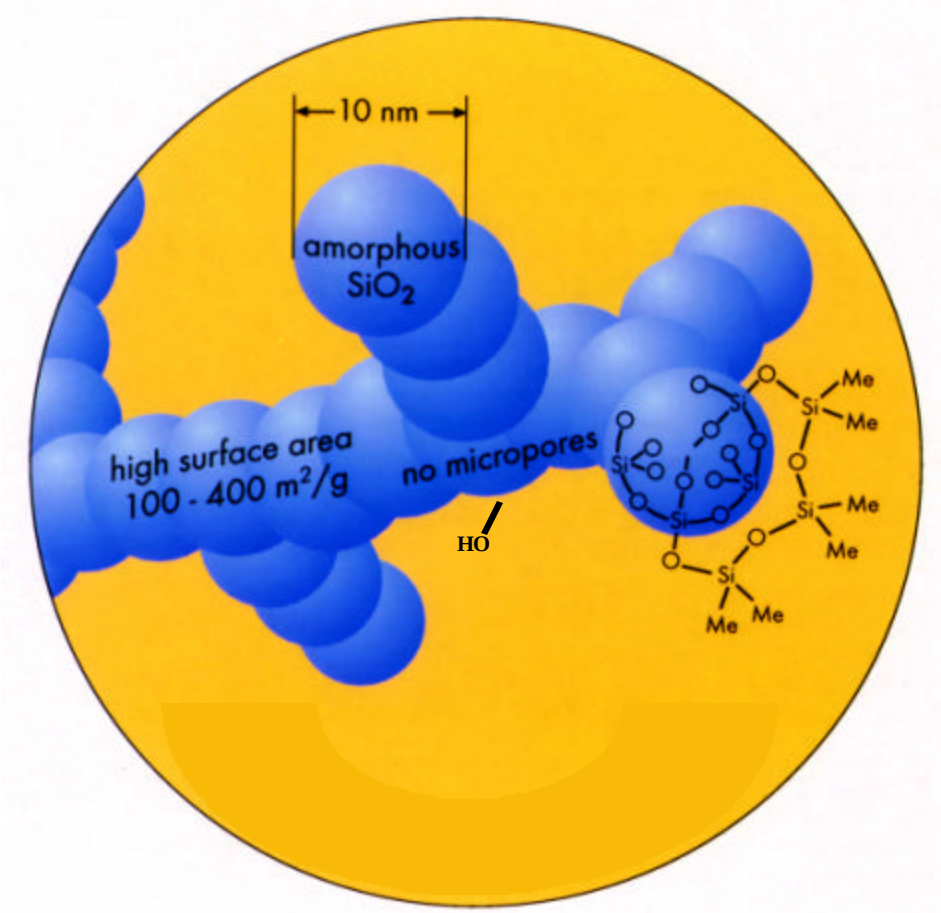
A schematic comparison of the surface structure of untreated hydrophilic amorphous silica and hydrophobic Silane, dichlorodimethyl-, reaction products with silica follows:

Figure 1 – untreated hydrophilic amorphous silica



Untreated (hydrophilic) amorphous silica has approximately 2 silanol groups (≡Si – OH) / nm^2.

Figure 2 – treated hydrophobic amorphous silica



Silane, dichlorodimethyl-, reaction products with silica has in addition to surface silanols, surface polydimethysiloxane (PDMS) units.

The core material is still amorphous silica

Comparison of basic physical-chemical properties

Property	Method	Hydrophilic silica	Hydrophobic silica Silane, dichlorodimethyl-, reaction products with silica
SiO_2 content	DIN 55921	≥ 99,8%	≥ 99,8%*
pH	DIN-ISO 787/9	3.5 - 4.5	3.5 - 4.5**
Carbon content		-	0.5 - 2 %
Color		white	white
Bulk density	DIN-ISO 787/11	50 g/l	50 g/l
Structure	X-ray	amorphous	amorphous

* based on the substance heated at 1000°C for 2 h

** based on a 1:1 mixture of water/methanol

The significant physicochemical similarity between the HPV Chemical Substance, Silane, dichlorodimethyl-, reaction products with silica and the non-surface treated (hydrophilic) synthetic amorphous silica lends itself to read across from the available reproduction / developmental toxicity data available for the latter to the former. The available reproductive / developmental toxicity data for untreated synthetic amorphous silica is summarized in the table below.

Test	Result	Remark
Fertility (one generation study, rat, oral) (1)	Parental toxicity: NOAEL = 497 mg/kg F1 offspring toxicity: NOAEL = 497 mg/kg	Only one dose was tested, no relevant effects were observed
Developmental toxicity / teratogenicity (rat, oral) (2)	Maternal toxicity: NOAEL = 1350 mg/kg Teratogenicity: NOAEL = 1350 mg/kg	No substance related effects up to the highest test dose
Developmental toxicity / teratogenicity (mouse, oral) (2)	Maternal toxicity: NOAEL = 1340 mg/kg Teratogenicity: NOAEL = 1340 mg/kg	No substance related effects up to the highest test dose
Developmental toxicity / teratogenicity (rabbit, oral) (2)	Maternal toxicity: NOAEL = 1600 mg/kg Teratogenicity: NOAEL = 1600 mg/kg	No substance related effects up to the highest test dose
Developmental toxicity/teratogenicity (hamster, oral) (2)	Maternal toxicity: NOAEL = 1600 mg/kg Teratogenicity: NOAEL 1600 mg/kg	No substance related effects up to the highest test dose

There is no evidence of reproductive toxicity associated with untreated synthetic amorphous silica, which is the core material of the HPV Chemical Substance, Silane, dichlorodimethyl-, reaction products with silica.

The incidence of testicular atrophy that was observed in one male in the two-year feeding study included in the initial IUCLID Data Set (30), is not indicated by the authors as being treatment related. It must also be noted that in two other sub-chronic studies using the test substance, there were no macro or microscopic effects on the testis (28, 34), epididymides or seminal vesicles (28).

Polydimethylsiloxane, which is formed on the surface of the untreated synthetic amorphous silica, has been tested for teratogenicity (rabbit, rat; subcutaneous) and reproductive performance (rat, primate; subcutaneous and dermal). These results are summarized in the ECETOC Joint Assessment of Commodity Chemicals Report No. 26.(4) There is no clear evidence of teratogenic effects or developmental toxicity after subcutaneous injection. The substance has no effect on fertility or gestation in the rat after subcutaneous administration. There is no indication of teratogenic or reproductive toxicity of polydimethylsiloxane after dermal or oral exposure.

In summary, there are no indications of reproductive effects with untreated synthetic amorphous silica, which is the core substance of the HPV Chemical Substance, Silane, dichlorodimethyl-, reaction products with silica. This is also true for the product itself and the polydimethylsiloxane which hydrophobes the surface of the untreated synthetic amorphous silica. We believe the weight of evidence associated with the available data does not support additional testing for reproductive endpoints. This is in line with animal welfare requirements (3-R principle).

TOXICITY TO FERTILITY

Type	:	One generation study
Species	:	rat
Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	oral feed
Exposure period	:	6 months
Frequency of treatm.	:	daily
Premating exposure period		
Male	:	4.5 month
Female	:	4.5 month
Duration of test	:	6 months
No. of generation studies	:	1
Doses	:	497 mg/kg (m); 509 mg/kg (f)
Control group	:	yes
NOAEL parental	:	= 497 mg/kg bw
NOAEL F1 offspring	:	= 497 mg/kg bw
Result	:	negative
Method	:	other: see Method
Year	:	1962
GLP	:	no
Method	:	Parents (40 m / 40 f), treatment started at a mean weight of 90 - 110 g; mating procedure (14 d): 5 treated and 5 control females (1 m to 5 f, resp.) after 4 1/2 months of exposure. The test-substance dose was adjusted to the body-weight gain. Haematology carried out in 5 animals of each group prior to exposure, each month and at the end of the study. Histopathology only in parent animals. Pups were examined for external appearance and development. Note: As compared to current standards, number of pregnant animals was too low (5 instead of 20), mating ratio was 1:5 instead of 1:2; one dose tested, not at the limit as recommended in the guideline 415. ---
Result	:	Parents: No clinical symptoms; no mortality, no abnormalities in body-weight gain and feed consumption, no haematological findings. In pups during lactation [total: 45 and 37 (control), resp.], no behavioural or developmental or structural abnormalities. ---
Test substance	:	Aerosil; Inventory Name: Silica, amorphous, fumed, cryst. free, CAS No. 112945-52-5
Reliability	:	(3) invalid 3a: Significant methodological deficiencies (no complete one generation study according to current standards: too low number of animals and examinations)
Flag	:	Critical study for SIDS endpoint

(1)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Wistar
Route of admin.	:	gavage
Exposure period	:	from day 6 to day 15 of gestation
Frequency of treatm.	:	daily
Duration of test	:	20 days
Doses	:	0, 13.5, 62.7, 292 and 1350 mg/kg bw/day
Control group	:	yes
NOAEL maternal tox.	:	= 1350 mg/kg bw
NOAEL teratogen.	:	= 1350 mg/kg bw
Method	:	
Year	:	1973
GLP	:	no
Result	:	The administration of up to 1350 mg/kg (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.

Test substance	:	Syloid 244: CAS Name: Silica gel, cryst.-free; CAS-No.: 112926-00-8
Reliability	:	(2) valid with restrictions 2e: Meets generally accepted scientific standards, well documented (though with deficiencies in description of test design) acceptable for assessment of mechanisms.
Flag	:	Critical study for SIDS endpoint

(2)

Species	:	mouse
Sex	:	female
Strain	:	CD-1
Route of admin.	:	gavage
Exposure period	:	from day 6 to day 15 of gestation
Frequency of treatm.	:	daily
Duration of test	:	20 days
Doses	:	0, 13.4, 62.3, 289 and 1340 mg/kg
Control group	:	yes
NOAEL maternal tox.	:	= 1340 mg/kg bw
NOAEL teratogen.	:	= 1340 mg/kg bw
Method	:	other
Year	:	1973
GLP	:	no
Result	:	The administration of up to 1340 mg/kg (body weight) of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.

Test substance	:	Syloid 244: CAS Name: Silica gel, cryst.-free; CAS-No.: 112926-00-8
Reliability	:	(2) valid with restrictions 2e: Meets generally accepted scientific standards, well documented (though with deficiencies in description of test design) acceptable for

(though with deficiencies in description of test design) acceptable for assessment of mechanisms.

Flag : Critical study for SIDS endpoint

(2)

Species	:	rabbit
Sex	:	female
Strain	:	Dutch
Route of admin.	:	gavage
Exposure period	:	from day 6 to day 18 of gestation
Frequency of treatm.	:	daily
Duration of test	:	29 days
Doses	:	0, 16.0, 74.3, 345 and 1600 mg/kg
Control group	:	yes
NOAEL maternal tox.	:	= 1600 mg/kg bw
NOAEL teratogen.	:	= 1600 mg/kg bw
Method	:	other
Year	:	1973
GLP	:	no
Result	:	The administration of up to 1600 mg/kg (body weight) of the test material to pregnant rabbits for 13 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.

Test substance	:	Syloid 244: CAS Name: Silica gel, cryst.-free; CAS-No.: 112926-00-8
Reliability	:	(2) valid with restrictions 2e: Meets generally accepted scientific standards, well documented (though with deficiencies in description of test design) acceptable for assessment of mechanisms.
Flag	:	Critical study for SIDS endpoint

(2)

Species	:	Syrian hamster
Sex	:	female
Strain	:	other: (outbred)
Route of admin.	:	gavage
Exposure period	:	from day 6 to day 10 of gestation
Frequency of treatm.	:	daily
Duration of test	:	14 days
Doses	:	0, 16.0, 74.3, 345 and 1600 mg/kg
Control group	:	yes
NOAEL maternal tox.	:	>= 1600 mg/kg bw
NOAEL teratogen.	:	>= 1600 - mg/kg bw
Method	:	other
Year	:	1973
GLP	:	no
Result	:	The administration of up to 1600 mg/kg (body weight) of the test material to pregnant hamsters for 5 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.

Test substance	:	Syloid 244: CAS Name: Silica gel, cryst.-free; CAS-No.: 112926-00-8
Reliability	:	(2) valid with restrictions 2e: Meets generally accepted scientific standards, well documented (though with deficiencies in description of test design) acceptable for assessment of mechanisms.
Flag	:	Critical study for SIDS endpoint

(2)

References:

(1) Degussa AG: Ueber die chronische Toxizität von AEROSIL. Unpublished report, LPT, Degussa AG - US-IT-No. 63-0001-DKT, 1963

(2) Food and Drug Research Laboratories, Inc. 1973: Teratologic Evaluation of FDA 71-48 (Syloid; silica aerogel). Reports prepared under DHEW Contract No. FDA 71-260. Maspeth NY [56 pp.]

(3) Lewinson, J., Mayr, W., Wagner, H.: Characterisation and Toxicological Behaviour of Synthetic Amorphous Hydrophobic Silica. Regul. Toxicol. Pharmacol., 20, 37-57, 1994

(4) European Centre for Ecotoxicology and Toxicology Of Chemicals (ECETOC); Joint Assessment of Commodity Chemicals (JACC) Report No. 26, Linear Polydimethylsiloxanes (CAS No.63148-62-9). Sep 1994